Original Article

Antifungal drug susceptibility of *Cryptococcus neoformans* from clinical sources in Nairobi, Kenya

Christine C. Bii,[1,2] Koichi Makimura,[3] Shigeru Abe,[3] Haruhiko Taguchi,[2] Olga M. Mugasia,[1] Gutura Revathi,[4] Njeri C. Wamae[1] and Shigeru Kamiya[2]

[1]*Mycology Laboratory, Center for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya,* [2]*Division of Medical Microbiology, Department of Infectious Diseases, Kyorin University School of Medicine, Tokyo, Japan,* [3]*Genome Research Centre and Institute of Medical Mycology, Teikyo University, Tokyo, Japan and* [4]*Kenyatta National Hospital, Nairobi, Kenya*

Summary

The serotypes and mating types of 80 clinical isolates of *Cryptococcus neoformans* from Kenya were studied and subjected to broth microdilution susceptibility testing to amphotericin B (AMP), flucytosin, fluconazole (FLC), itraconazole (ITC) and miconazole (MCZ). The isolates included *C. neoformans* var. *grubii* – 75 of 80 (serotype A; 93.7%), *C. neoformans* var. *neoformans* – three of 80 (3.8%) and *C. neoformans* var. *gattii* – two (serotype B; 2.5%). Mating experiment confirmed all the isolates to be α-mating type. Seventy-eight (97.5%) of the isolates had minimum inhibitory concentration (MIC) of ≤0.5 μg ml^{-1} to AMP and at 1 μg ml^{-1}, 100% of the isolates were inhibited. Flucytosin resistance was observed in 21% with MIC in which 90% of the isolates were inhibited (MIC_{90}) of 64 μg ml^{-1}. Only 23.8% of the strains were susceptible to FLC with 65% susceptible dose-dependent (SDD) and 11.2% resistant. Itraconazole susceptibility was 61.3% while the rest were either SDD or resistant. The MIC_{90} for ITC and MCZ were 0.5 and 2 μg ml^{-1} respectively.

The study reports the serotypes, mating types and highlights the existence of azoles resistance in *C. neoformans* in Nairobi which calls for antifungal drug resistance surveillance as prophylactic use of FLC increases because of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) epidemic in sub-Saharan Africa.

Key words: serotypes, resistance, *Cryptococcus neoformans*, Kenya.

Introduction

Human immunodeficiency virus (HIV) epidemic has led to a growing population of immunocompromised patients at risk of contracting opportunistic fungal infections particularly cryptococcosis.[1] Globally, the risk for cryptococcal meningitis HIV/acquired immunodeficiency syndrome (AIDS) is estimated at 6–8% in adults and 1% in children.[2,3] In sub-Saharan Africa which has the highest burden of HIV/AIDS worldwide, the incidence of meningoencephalitis has increased significantly with mortality higher than meningococcal meningitis caused by *Neisseria meningitidis*.[4,5]

Cryptococcus neoformans consist of serotypes, A, B, C, D and AD classified into three varieties: *C. neoformans* var. *neoformans* (serotype D), *C. neoformans* var. *grubii* (serotype A) both of the teleomorph *Filobasidiella neoformans* var. *neoformans* and *C. neoformans* var. *gattii* (serotypes B and C) of the teleomorph *F. neoformans* var. *bacillisporus*.[6,7] The prevalence and geographical distribution of the two varieties vary. Generally, *C. neoformans* var. *neoformans* is the most prevalent with worldwide distribution while *C. neoformans* var. *gattii* is limited to tropics and subtropical regions coinciding with the distribution of the host trees *Eucalyptus camaldulensis* and *E. tereticornis*.[8] Distinct genotype clusters among isolates of African, America and Europe

Correspondence: Christine C. Bii, Mycology Laboratory, Kenya Medical Research Institute, PO Box 2632, 00202 Nairobi, Kenya.
Tel: 254 2724264. Fax: 254 2720030.
E-mail: kiptobi@yahoo.com

Accepted for publication 26 June 2006

  doi:10.1111/j.0933-7407.2006.01293.x

origin may exist[9–11] but whether this is clinically relevant in terms of drug susceptibility and virulence-related factors remain unclear.

Despite the World Health Organization (WHO) initiatives on antimicrobial resistance surveillance, antifungal drug resistance surveillance in sub-Saharan Africa remains somewhat ignored. While improvement in susceptibility to antifungal drugs was recently reported in Europe and the USA,[12] the need for life-long fluconazole (FLC) maintenance therapy due to high relapse rates of cryptococcosis in HIV/AIDS raises concerns over antifungal resistance in developing countries.[13,14] As FLC becomes widely used due to expanding population of HIV/AIDS confounded by irrational use of antibiotics, and generic antibiotics in developing countries, emergence azole resistance cannot be inevitably ignored as a mycological challenge.[15] The study was undertaken to characterise *C. neoformans* serotypes and antifungal drug susceptibilities essential for a mounting appropriate clinical management for cryptococcal infection in HIV/AIDS in Kenya.

Materials and methods

Cryptococcus strains

Eighty *C. neoformans* isolates from cerebral spinal fluids (CSF) specimens of adult patients hospitalised with cryptococcal meningitis from two referral hospitals in Nairobi, Kenya were used in the study. The specimens were collected from individual patients before therapy was initiated. Between January 2003 and January 2004 72 strains from HIV-positive patients and eight from patients with unknown HIV status were isolated at Mycology Laboratory, Kenya Medical Research Institute. Primary isolation was underwent on Sabourauds dextrose agar incubated at 30 °C for 72 h and preliminary identification was performed by demonstration of capsule on Indian ink.

Biochemical and immunological characterisation

All the isolates were subcultured on CHROMagar Candida (CHROMagar, Paris, France) to rule out contamination and to ensure purity of the isolates by its ability to discriminate *Candida* spp. Confirmation of *C. neoformans* was carried out by Vitek Yeast Biochemical Cards (bioMerieux-Vitek, Hazelwood, MO, USA). Serotypes were determined by slide agglutination test using Crypto Check agglutination kit (Iatron Labs Inc., Tokyo, Japan).

Mating experiment

The mating types (MAT) were determined as described previously.[7] Authentic strains: *C. neoformans* TLD-350 serotype A, MATα, *C. neofomans* TLD-261 serotype D, MATα, *C. neoformans* TLD-262 serotype D, MAT*a*, *C. neoformans* TLD-263 serotype B, MATα and *C. neoformans* TLD-264 serotype C, MATα were used. Briefly, individual isolates were co-cultured with the tester strain in an agar medium containing KH_2PO_4, $MgSO_4$, $CaCl_2$, NaCl, biotin, sucrose and yeast extract and incubated at 30 °C for 20 days. Mating was considered successful when mycelia with characteristic clamp connection were observed.

Antifungal drug susceptibility

Cryptococcus isolates were subjected to broth microdilution susceptibility test using commercial Frozen Plate Kit (Eiken Chemical Co., Ltd, Tokyo, Japan).[16] The Kit contains the following drug dilution ranges: amphotericin B (AMP) 0.03–16 μg ml^{-1}, flucytosin (5FC) 0.125–64 μg ml^{-1}, FLC 0.125–64 μg ml^{-1}, itraconazole (ITC) 0.015–8 μg ml^{-1}, miconazole (MCZ) 0.06–32 μg ml^{-1} and micafungin (MCFG) 0.03–16 μg ml^{-1}. The Kit has been evaluated in a multicentre study with over 90% agreement with CLSI.[17,18] The procedures and minimum inhibitory concentrations (MIC) were carried out and interpreted according to the manufacturer's instructions. The MIC for azoles and 5FC were scored as the lowest drug concentration that resulted in 80% growth inhibition (IC_{80}) while 100% reduction of turbidity was considered MIC for AMP. Quality control was performed using *Candida krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 and results excepted only if the MIC were within the recommended range.

The study protocol was approved by the Kenya Medical Research Institute ethical and scientific steering committee (SSC) before implementation and was assigned SSC number 766. The procedures conformed to all the scientific and ethical standards during its implementation.

Results

Identification and typing

Seventy-five of 80 (93.7%) isolates were identified as *C. neoformans* var. *grubii* (serotype A). Only three isolates were identified as *C. neoformans* var. *neoformans* (two serotype AD and one serotype D) while two

isolates were identified as *C. neoformans* var. *gattii* (serotype B); however, all the isolates were identified as MATα.

Drug susceptibility

All the isolates were susceptible to AMP with MIC_{90} of 0.5 μg ml^{-1} and only two isolates with MIC of 1 μg ml^{-1} (Table 1). All except one isolate had MIC beyond susceptible level (MIC ≤4 μg ml^{-1}) to 5FC while 77.6% of the isolates had MIC ranging from 8 to 32 μg ml^{-1}. Resistance (MIC ≥ 64 μg ml^{-1}) to 5FC was 21.2%. The MIC_{90} for 5FC and FLC was 64 μg ml^{-1} each. Nine of 80 (11.2%) isolates were resistant (MIC ≥64 μg ml^{-1}) to FLC while 52 of 80 (65.0%) were categorised as susceptible dose-dependent (SDD; MIC 16–32 μg ml^{-1}). Among the azoles tested, ITC had the lowest MIC_{90} similar (0.5 μg ml^{-1}) to that of AMP; however, there were three isolates with MIC ≥8 μg ml^{-1} to ITC. Only seven of 80 (8.8%) of the isolates were fully susceptible (MIC ≤0.125 μg ml^{-1}) to MCZ, 62 of 80 (77.5%) were SDD (MIC 0.25–1 μg ml^{-1}) and 11 of 80 (13.7%) were resistant (MIC >1 μg ml^{-1}). The MIC_{90} for MCZ was 2 μg ml^{-1}. The only isolate identified as serotype D exhibited MIC beyond susceptible range to all except AMP with MICs (μg ml^{-1}) as follows: AMP 0.5, 5FC >64, FLC >64, ITC >8 and MCZ 8. All the isolates tested were resistant (MIC ≥16 μg ml^{-1}) to MCFG (data not shown).

Discussion

Cryptococcus neoformans var. *grubii* (serotype A) MATα was the predominant isolate with evidence of azole resistance. This is in consistent with other reports of the worldwide distribution of this serotype and its predilection for HIV/AIDS.[1] Two isolates of serotype AD and one serotype D were identified indicating the infrequent cause of these serotypes in cryptococcoses in accord with reports that African patients are rarely infected with *C. neoformans* var. *neoformans* serotype D.[19] Two strains of *C. neoformans* var. *gattii* (serotype B) were identified, although this serotype rarely infects HIV/AIDS patients, the two strains were recovered from patients whose HIV status was unavailable. *Cryptococcus neoformans* var. *gattii* is predominant in tropical and subtropical areas coinciding with the distribution of the host tree, the *Eucalyptus* species.[8,20] These trees are now widely grown in Kenya for its timber products and that could have an influence on the presence of this serotype in Kenya. Nonetheless, the study confirms the existence of *C. neoformans* var. *gattii* (serotype B) in Kenya and it would be important to ascertain its clinical significance.

Despite evidence of sexual recombination among *C. neoformans* in sub-Saharan Africa,[21] all the strains were identified as MATα-type, which further supports the hypothesised predominance of MATα over MAT*a* in both clinical and natural environment.[22] The prevalence and virulence of *C. neoformans* has been linked to mating types. Pathogenic strains are largely asexual[23]

Table 1 Antifungal drug susceptibility of *Cryptococcus neoformans* isolates from Kenya

Antifungal drug concentration (μg ml^{-1})	Number of susceptible isolates (%)				
	Amphotericin B	Flucytosin	Fluconazole	Itraconazole	Miconazole
0.06	0 (0)	0 (0)	0 (0)	29 (36.3)	5 (6.2)
0.125	10 (12.5)	0 (0)	0 (0)	20 (25.0)	2 (2.5)
0.25	27 (33.8)	0 (0)	0 (0)	19 (23.8)	15 (18.8)
0.5	41 (51.2)	1 (1.2)	0 (0)	7 (8.8)	35 (43.8)
1	2 (2.5)	0 (0)	1 (1.2)	2 (2.5)	12 (15.0)
2	0 (0)	0 (0)	3 (3.8)	0 (0)	5 (6.2)
4	0 (0)	0 (0)	3 (3.8)	0 (0)	4 (5.0)
8	0 (0)	15 (18.8)	12 (15.0)	3 (3.8)	2 (2.5)
16	0 (0)	23 (28.8)	46 (57.5)	–	0 (0)
32	–	24 (30)	6 (7.5)	–	0 (0)
64	–	17 (21.2)	9 (11.2)	–	–
MIC_{50} (%)	0.5	64	16	0.125	0.5
MIC_{90} (%)	0.5	64	64	0.5	2
MIC of *Candida parapsilosis* ATCC 22019	0.25	0.5	0.5	0.25	2
MIC of *Candida krusei* ATCC 6258	1	16	32	0.25	2

MIC_{90}: concentration at which 90% of the isolates were inhibited.
Horizontal line show beyond dilution range tested.

and unlike MAT*a*, the MATα strains posses a 55 kb gene locus responsible for virulence.[24] Although MAT*a* strains have been reported in Tanzania,[22] it was not detected in our study.

All the isolates were susceptible to AMP (MIC_{90} 0.5 μg ml^{-1}) in accord with other reports that the MIC to AMP has remained unchanged over time with no evidence of resistance.[12] Although the MIC_{90} was lower than that reported for Malawi (0.5 μg ml^{-1} vs. 2 μg ml^{-1}), it further complement reports of isolated strains from African with elevated MIC to AMP[25,26] and calls for continued surveillance.

Resistance to 5FC was unusually high (21.2%) with majority (77.6%) of the isolates having MIC between 8 and 32 μg ml^{-1} in contrast with previous reports of high susceptibility and no instances in which increased MIC to 5FC has been reported.[12] Despites, reports that antifungal susceptibility has improved worldwide, pockets of resistance cannot be ruled out as an exhaustive surveillance has not been conducted in areas where resistance has previously been reported. Moreover, there are emerging reports that the epidemiology and drug susceptibility of *C. neoformans* strains from some African countries are different which are possible pockets of resistance.[11,27,28] Although the frequency of 5FC and azole resistance was unusually high, due to lack of proper follow up and ethical reasons we could not link up susceptibility and clinical data to ascertain whether patients infected with these resistant strains had poor prognosis. We hope this will address in our newly proposed study which will also collect more of such isolates to be tested using CLSI broth microdilution technique.

Fluconazole resistance was detected at a frequency of 11.2% while the MIC_{90} of ITC and MCZ were 0.5 and 2 μg ml^{-1} respectively. In Spain, reduced susceptibility to FLC and 5FC has been reported with resistance of 5.3% and 15.8% to AMP and ITC while decreased susceptibility to azoles associated with fungal burden and immunosuppression has been reported.[29,30] Similar pattern have been reported in Cambodia and other developing countries and is associated with FLC maintenance therapy in AIDS.[11,31,32] While an overall shift towards susceptibility to FLC and 5FC attributed to decrease incidence of cryptococcosis and effective antiretroviral programmes in the USA and Europe may be true, this is unlikely in developing countries particularly sub-Saharan Africa where the prevalence of HIV/AIDS and crytococcal meningitis continue to increase. Susceptibility to FLC has been shown to improve in AIDS patients on highly active antiretroviral therapy (HAART),[33] while this may be the case in USA and Europe, unfortunately <1% of AIDS patients in sub-Saharan African are on HAART.

The reasons for the low susceptibility to FLC were not apparently clear in our study but it is notable that the isolates were recovered from AIDS patients from an urban referral hospital. Being a referral hospital majority of the patients were likely to have been previously exposed to FLC or were relapse cases this; however, could not be confirmed in our study. Reduced susceptibility to FLC has been noted in AIDS and in patients with recurrent meningitis. Furthermore, it has been demonstrated that serial isolates of *C. neoformans* from AIDS patients have altered virulence-related factors and drug susceptibilities.[14,15] However, we further speculate that poor prescription practices and under dosages may be confounding factors. Although earlier studies show improved antifungal susceptibility it does not indicate whether the strains included those from Kenya or sub-Saharan African with the highest burden of HIV/AIDS. Improved susceptibility in developed countries is possible due to accessibility and affordability of other effective triazoles, declining rates of HIV/AIDS and cryptococcoses, effective HAART programmes and good prescription practices which is not the case in sub-Saharan Africa. The hidden dangers of FLC maintenance therapy in HIV/AIDS in poor resource countries in sub-Saharan Africa cannot still be under estimated.

Clinical response to fluconzole is likely only when the MIC is <16 μg ml^{-1}[34] and FLC resistance rate of 11.2% for a developing country like Kenya is intriguing and may be a reflection of poor prognosis currently witnessed in patients with meningoencephalitis in Kenya. As FLC becomes increasingly used due to the need for life-long maintenance therapy in HIV/AIDS patients, we recommend measures to control irrational use of antifungal drugs and establishment of constant antifungal drug resistant surveillance in sub-Saharan Africa where emerging resistance is more likely.

Acknowledgments

This investigation on Research and Control of Opportunistic Infections was supported by grants from the Japanese International Cooperation Agency (JICA) and Kenya Medical Research Institute (KEMRI). Authentic strains were kindly provided by Dr R. Kano and Professor A. Hasegawa of Nihon University School of Veterinary Medicine.

This work is published with the permission of the Director KEMRI.

Reference

1 Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS-100 years after the discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev* 1995; **8**: 515–48.

2 Steenbergen JN, Casadevall A. Prevalence of *Cryptococcus neoformans* var. *neoformans* (serotype D) and *Cryptococcus neoformans* var. *grubii* (serotype A) isolates in New York City. *J Clin Microbiol* 2000; **32**: 1974–1976.

3 Goldman DL, Khine H, Abadi J *et al.* Serological evidence of *Cryptococcus neoformans* infection in early childhood. *Pediatrics* 2001; **107**: e66.

4 Bogaerts J, Rouvroy D, Taelman H *et al.* AIDS-associated cryptococcal meningitis in Rwanda (1983–1992): epidemiologic and diagnostic features. *J Infect* 1999; **39**: 32–7.

5 Heyderman RS, Gangaidzo IT, Hakim JG *et al.* Cryptococcal meningitis in human immunodeficiency virus-infected patients in Harare, Zimbabwe. *Clin Infect Dis* 1998; **26**: 284–9.

6 Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans* var. *grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. *J Clin Microbiol* 1999; **37**: 838–40.

7 Kwon-Chung KJ. A new species of *Filobasidiella*, the sexual state of *Cryptococcus neoformans* B and C serotypes. *Mycologia* 1976; **68**: 942–5.

8 Kwong-Chung KJ, Bennett JE. High prevalence of *Cryptococcus neoformans* var. *gattii* in tropical and subtropical regions. *Zentralbl Bakteriol Mikrobiol Hyg A* 1984; **257**: 213–8.

9 Nielsen K, Cox GM, Wang P *et al.* Sexual cycle of *Cryptococcus neoformans* var. *grubii* and virulence of congenic *a* and α isolates. *Infect Immun* 2003; **71**: 4831–41.

10 Boekhout T, Theelen B, Diaz M *et al.* Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans*. *Syst Evol* 2001; **147**: 891–907.

11 Chandenier J, Adou-Bryn KD, Douchet C *et al.* In vitro activity of amphotericin B, fluconazole and voriconazole against 162 *Cryptococcus neoformans* isolates from Africa and Cambodia. *Eur J Clin Microbiol Infect Dis* 2004; **23**: 506–8.

12 Pfaller MA, Messer SA, Boyken L *et al.* Global trends in the antifungal susceptibility of *Cryptococcus neoformans* (1990 to 2004). *J Clin Microbiol* 2005; **43**: 2163–7.

13 Pfaller MA, Zhang J, Messer SA *et al. In vitro* activities of vericonazole, fluconazole and itraconazole against 566 clinical isolates of *Cryptococcus neoformans* from United States and Africa. *Antimicrob Agents Chemother* 1999; **43**: 169–71.

14 Paugam A, Blanche DP, Gangnenux JP, Tourte-Schaefer C, Sicard D. Increased fluconazole resistance of *Cryptococcus neoformans* isolated from patients with AIDS and recurrent meningitis. *Clin Infect Dis* 1994; **19**: 975–6.

15 Berg J, Clancy CJ, Nguyen MH. The hidden dangers of primary fluconazole prophylaxis for patients with AIDS. *Clin Infect Dis* 1998; **26**: 186–7.

16 Makimura K, Suzuki T, Tamura T *et al.* Comparative evaluation of standard dilution method and commercial kit for frozen plate antifungal susceptibility testing of yeasts using 200 clinical isolates. *Microbiol Immunol* 2004; **48**: 747–53.

17 National Committee for Clinical Laboratory Standards. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard*, 2nd edn, Document M27-A2. Wayne, PA: National Committee for Clinical Laboratory Standards, 2002.

18 Makimura K, Oguri T, Mikami Y *et al.* Multicenter evaluation of commercial frozen plates for microdilution broth antifungal susceptibility testing of yeasts and comparison of MIC limits recommended in NCCLS M27-A2. *Microbiol Immunol* 2005; **49**: 97–106.

19 Garcia-Hermaso D, Janbon G, Drome F. Epidemiological evidence for dormant *Cryptococcus neoformans* infection. *J Clin Microbiol* 1999; **37**: 3204–9.

20 Ellis DH, Pfeiffer TJ. Natural habitat of *Cryptococcus neoformans* var. *gattii*. *J Clin Microbiol* 1990; **28**: 1642–4.

21 Litvintseva AP, Marra RE, Nielsen K *et al.* Evidence of sexual recombination among *Cryptococcus neoformans* serotype A isolates in sub-Saharan Africa. *Eukaryot Cell* 2003; **2**: 1162–8.

22 Lengeler KB, Wang P, Cox GM, Perfect JR, Heitman J. Identification of the MATa mating-type locus of *Cryptococcus neoformans* reveals a serotype A MAT*a* strain thought to have been extinct. *Proc Natl Acad Sci USA* 2000; **97**: 14455–60.

23 Nielsen K, Cox GM, Wang P *et al.* Sexual cycle of *Cryptococcus neoformans* var. *grubii* and virulence of congenic α and alpha isolates. *Infect Immun* 2003; **71**: 4831–41.

24 Chang YC, Winkers BL, Miller GF, Penoger LA, Kwon-Chung KJ. *Cryptococcus neoformans* STE12α regulates virulence but is not essential for mating. *J Exp Med* 2000; **191**: 871–82.

25 Neil F, Katherine G, Watera C *et al. Cryptococcus* infection in a cohort of HIV-1-infected Ugandan adults. *AIDS* 2000; **16**: 1031–8.

26 Powderly WG, Keath EJ, Sokol-Anderson M *et al.* Amphotericin B-resistant *Cryptococcus neoformans* in a patient with AIDS. *Infect Dis Clin Pract* 1992; **1**: 314–6.

27 Archibald LK, Tuohy MJ, Wilson DA *et al.* Antifungal susceptibilities of *Cryptococcus neoformans*. *Emerg Infect Dis* 2004; **10**: 143–5.

28 Pfaller M, Zhang J, Messer S *et al.* Molecular epidemiology and antifungal susceptibility of *Cryptococcus neoformans* isolates from Uganda AIDS patients. *Diagn Microbiol Infect Dis* 1998; **32**: 191–9.

29 Cuenca-Estrella M, Diaz-Guerra TM, Mellado E, Rodriguez-Tudela JL. Flucytosin primary resistance in *Candida* species and *Cryptococcus neoformans*. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 276–9.

30 Perkins A, Gomez-Lopez A, Mellodo E, Rodriguez-Tudela JL, Guenca-Estrella M. Rates of antifungal resistance

among Spanish clinical isolates of *Cryptococcus neoformans* var. *neoformans*. *J Antimicrob Chemother* 2005; **56**: 1144–7.

31 Sar B, Monchy D, Vann M, Keo C, Sarthou JL, Buisson Y. Increasing in vitro resistance to fluconazole in *Cryptococcus neoformans* Cambodian isolates: April 2000 to March 2002. *J Antimicrob Chemother* 2004; **54**: 563–5.

32 Datta K, Jain N, Sethi S, Rattan A, Casadevall A, Banerjee U. Fluconazole and itraconazole susceptibilities of clinical isolates of *Cryptococcus neoformans* at a tertiary care centre in India; a need for care. *J Antimicrob Chemother* 2003; **52**: 683–6.

33 Martins MD, Lozano-Chiu M, Rex JJ. Declining rates of oropharyngeal candidiasis and carriage of *Candida albicans* associated with trends toward reduced rates of carriage of fluconazole-resistant *C. albicans*. *Clin Infect Dis* 1998; **27**: 1291–12.

34 Aller AI, Martin-Mazuelos E, Lozano F *et al.* Correlation of fluconazole MICs with clinical outcome in cryptococcal infection. *Antimicrob Agents Chemother* 2000; **44**: 1544–8.